REVIEW

THE DISSERTATION ON TOPIC: " CATALASE FROM ANTARCTIC FUNGI: ROLE IN ANTIOXIDANT DEFENSE, REGULATION AND PROPERTIES"

Professional direction 4.3. Biological Sciences (Microbiology)

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Doctoral student Vladislava Dishliyska acquires a Master's degree at the Faculty of Biology at SU "St. Kliment Ohridski" in 2001, majoring in Biotechnological processes with a specialization in "Industrial Biotechnologies". She joined the Institute of Microbiology (BAS) in 2005; at the end of 2007, she was appointed as an assistant in the "Mycology" section, where she still works today. On 27.04.2022, she enrolled as a doctoral student, receiving free training.

The topic of the current thesis project is the study of the role of enzymes with catalase activity in strains of filamentous fungi belonging to different temperature classes in the morphological, metabolic, and genetic mechanisms of adaptation to low temperature stress.

The presented dissertation is structured according to established practice, within 117 standard pages, of which the cited literature occupies 27 pages and includes 306 citations. The presented development is very well illustrated with 12 tables and 30 figures.

Microorganisms inhabiting regions with extreme climatic conditions are the center of attention of world science. There is a growing interest and database on life and biodiversity in Antarctica, which is one of the most well-preserved and clean areas on the planet, but with very harsh living conditions. The ability of Antarctic microorganisms to exist in some of the harshest climates known on earth has led to increased interest in their metabolism. Studying and characterizing the species diversity in this region, as well as their metabolic capabilities, can provide new knowledge and solutions for industry and environmental protection.

Fungi are among the most widespread microorganisms in these areas due to their rich enzyme apparatus, which allows them to be highly adaptable in conditions of lack of standard nutrients, low temperatures, drought, etc.

One of the most interesting and up-to-date studies in this context is related to the mechanisms of antioxidant protection. These mechanisms are still poorly and insufficiently studied in filamentous fungi, especially in conditions of low temperatures close to negative.

Oxidative stress is caused by an imbalance between the production and accumulation of so-called free oxygen radicals (ROS) in the cells and tissues of aerobic organisms and the ability of the relevant biological system to detoxify these reactive products. ROS are known to be commonly generated as byproducts of oxygen metabolism and play an important physiological role. In addition to long-proven environmental factors causing a significant increase in ROS production, low temperatures have also been shown to cause more free oxygen radicals, damaged protein molecules, spare carbohydrates, oxidized lipids, and damaged nucleic acids, resulting in oxidative stress.

To protect against ROS-induced cell damage, cells have an antioxidant defense system based mainly on enzymes, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). One of the important indicators of the presence of oxidative stress is the increase in the activity of these enzymes, as well as the accumulation of reserve carbohydrates trehalose and glycogen.

The literature review contains both a historical overview and new and interesting information related to the topic under consideration. Demonstrates excellent knowledge of scientific achievements and developments in the field of the dissertation topic. The components and mechanisms of antioxidant protection are very clearly described. Central attention is given to the characteristics and vital role of the known groups of catalase enzymes. Focus is placed on the features of temperature-sensitive catalases. An important place is also devoted to the filamentous fungi found on Antarctica.

The unsolved problems formulated at the end of the overview related to the topic of the dissertation being developed point to the author's desire to contribute to their solution.

The purpose clearly reflects the essence of the dissertation work. For its implementation, 7 main research tasks have been defined, the third of which has three sub-points. The specific course of research is well outlined.

In the chapter "Materials and Methods" the classical and state-of-the-art analysis approaches used in the scientific research are detailed, covering cultivation methods, electron microscopic (TEM and SEM), spectrophotometric analysis in the determination of specific enzyme activity of antioxidant enzymes and determination of the production of H_2O_2, isolation of DNA and RNA, Polymerase chain reaction (PCR), quantitative PT PCR amplification, DNA sequence analysis and bioinformatics analysis of the sequenced oligonucleotides, purification of temperature-sensitive catalase, etc.

In the initial parts of the chapter "Results and Discussion" data obtained as a result of the so-called "routine" microbiological work, which, however, requires a lot of experience,

effort, and patience. As a result of the research on the influence of different temperatures on the growth and development of 61 strains of Antarctic filamentous fungi, their belonging to different thermal classes was determined. The ability of each of the studied strains to synthesize intracellular and/or extracellular catalase was determined by enzymatic studies. The corresponding enzyme activity was also determined. Data for 31 of the studied strains are presented.

As a result of a comparative analysis of the results of these studies, two model strains belonging to different temperature classes—the psychrotolerant *Penicillium griseofulvum* P29 and the mesophilic *Penicillium chrysogenum* P27—were selected for further research.

At this stage, the changes in their growth, development, and sporulation, the levels of generated COPs, oxidative damaged protein molecules, and reserve carbohydrates, as well as the degree of membrane integrity under the influence of low temperatures, were studied. The activity of SOD and CAT enzymes from the antioxidant defence of the two strains was also determined. The results prove that the accumulation of COP is higher in *P. chrysogenum* strain P27. Strain *P. griseofulvum* P29 showed a lower degree of oxidative damage to proteins and lipids.

A difference between the two strains was also observed regarding the activation of the studied enzymes. The results outline a more pronounced increase in SOD for the mesophilic strain, while the activation of the antioxidant enzyme defense in the psychrotolerant strain is due to a greater extent to the increased CAT activity.

Based on all these results, PhD student Vladislava Dishlijska came to the conclusion that strain *P. griseofulfum* P29 is not only a better producer of the enzyme catalase but is a potential producer of thermosensitive enzymes. The following in-depth studies are focused on it.

Of particular interest are the results obtained through the conducted electron microscopic analyses (Scanning Electron Microscopy and Transmission Electron Microscopy). The ultrastructural changes in the cells of strain *P. griseofulvum* P29 were monitored upon application of 6 hours of stress at 15°C and at 6°C. At both temperatures, alterations are observed in the various cell organelles. Disturbed density of the cell wall, localization of eisosome-like structures, changes in the surface and size of hyphae, etc. are observed. The extent of degenerative disorders is much more pronounced at the lower temperature.

Unique information about the genetic potential of strain *P. griseofulvum* P29 as a catalase producer was provided by subsequent molecular analyses to identify and sequence

the genes encoding proteins with catalase activity in the model strain. Phylogenetic analyses of fungal catalases show that the number of enzymes varies among species. Comparative analysis of the obtained complete genome sequence of strain *P. griseofulvum* P29 has shown the possible presence of 5 different catalase genes, which have been identified, sequenced, and characterized in terms of the functionality of their encoded proteins. Following the expression of these genes in the conditions of two temperature regimes (10°C and 25°C), with the help of RT-qPCR, the increase was also proven at the lower temperature. The strongest increase in the expression of the cat1 gene, encoding the catalase-peroxidase enzyme, suggests its important role in adaptation processes and cell survival in conditions of low-temperature oxidative stress.

The following experiments are aimed at creating conditions for maximal expression and production of temperature-sensitive catalase. Optimum values of glucose, carbon source, inoculum concentration, temperature, and duration of cultivation were established. The percentage of dissolved oxygen in the medium was found to have the greatest impact. As the level of RK increases to 40%, the enzyme activity increases to the maximum, 77.5 U/mg, achieved after 84-96 hours of cultivation. This is an important stage in the development since such studies, especially on the influence of RK, are practically not presented in the scientific literature. Information on the synthesis and properties of CA CAT is very scarce. There are known studies in some species of bacteria, but in fungi studies are practically absent. In conclusion of the research, it can be claimed that the Antarctic strain *P. griseofulvum* P29 is an original effective fungal producer of CA CAT. The performance of *P. griseofulvum* strain P29 in a high-volume bioreactor was demonstrated. The strain synthesizes intracellular and extracellular catalase in a ratio of 8:1.

Subsequent application of an efficient CAT purification protocol resulted in a 12-fold increase in specific activity to 538 E/mg b, with the yield decreasing about 11-fold. Studies were conducted with the purified catalase enzyme to determine the conditions (temperature, pH) for optimal activity and stability of the resulting enzyme preparation. The obtained results are important both for the continuation of fundamental research with temperature-sensitive fungal catalases and for the development of various industrial processes, such as the neutralization of residual bleaching chemicals in the textile industry, for improving the quality of food products and packaging materials by removing H_2O_2, as a potential ingredient of pharmacological formulations, etc.

As a result of the obtained data, Vladislava Dishliyska formulated 9 conclusions and 4 contributions, adequately reflecting the results obtained during the development of the

dissertation work. The assigned tasks have been fully completed. Here is the place to emphasize the competence of the scientific leader, Associate Professor Krumova, as well as the entire team of the Mycology Department, who have been successfully working on topics closely related to the mechanisms of oxidative stress for years.

I have no objections to the development submitted for defense, as the recommendations made to the internal defense are reflected in the version submitted for review.

CONCLUSION

Studies on temperature-sensitive catalases are of utmost importance for elucidating the metabolism of psychrophilic and psychrotolerant microorganisms. Since fungi are eukaryotes, the conclusions obtained on their basis can be of general biological importance.

The problems posed in the presented development are original, current, and significant, mainly for the field of fundamental research but also with regard to relevant scientific and applied developments. Formulated conclusions and contributions are well-argued, with original character and scientific significance.

During the development of this dissertation, Vladislava Dishliyska has earned 272 credits, which is significantly higher than the required minimum. Parts of the results included in the doctoral development were published in 2 articles in prestigious international publications, with a total IF of 5,532. On the subject of the dissertation, two reports were given at two scientific forums.

Doctoral student Vladislava Dishlijska has used a rich arsenal of classical and modern analytical, biochemical, microbiological, and molecular genetic methods, which has made her a highly qualified, methodologically prepared specialist.

The presented dissertation on the topic "Catalase from Antarctic mushrooms: role in antioxidant protection, regulation, and properties" fully corresponds in volume and significance of the experimental activity and obtained results to the scientific and legal requirements in Bulgaria. My assessment of the presented dissertation work is entirely positive. It gives me a full reason to recommend to the respected members of the Scientific Jury to vote for awarding doctoral student Vladislava Dishliyska with the scientific degree "Doctor" in the field of Professional Direction 4.3. Biological Sciences (Microbiology).

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/Assoc. Dr. Zlatka Alexieva/